QTL Analysis Of Vitiligo In An Outbred Chicken Intercross

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Introduction

Vitiligo is an autoimmune disease characterized by pigment loss in the skin due to the death of melanocytes. Evidence for an autoimmune origin is the observation of T-cells in active lesions in some, but not all, of the patients and the fact that vitiligo has a close association to other autoimmune diseases (autoimmune thyroid disease, pernicious anemia, Adisson's disease and alopecia areata) (Spritz, 2005). 1-2% of the human population is affected, which makes it one of the most common pigmentary disorders. Genetic, environmental and immunologic factors all contribute to destruction of melanocytes, which makes vitiligo a truly complex disease. Lack of good laboratory animal models have inhibited the progress of getting a clear clinical definition of vitiligo as well as the complex genetic architecture underlying the disease (Spritz, 2005). In this paper we have used the Smyth Line (SL) chickens developed at the University of Massachusetts as an animal model for the disease. 70-95% of the SL chickens are affected by vitiligo with several features similar to those found in humans and are therefore valuable animal models to study autoimmune vitiligo in humans.

Material and methods

Animals. We have studied an F_2 cross between Smyth Line and Brown Line (control) chickens. 14 SL chickens were crossed with 12 Brown Line (BL) chickens. 57 birds from this F_1 generation was thereafter intercrossed to produced over 500 F_2 chickens, from which 496 birds was used in the analysis. Only animals with genotypes assigned with more than 80% probability were included in the analysis. Individuals were classified according to the most probable genotype (i.e. homozygote or heterozygote) for each locus.

Methods. Vitiligo was phenotyped as a binary trait, healthy or sick. Birds were genotyped for 348 markers covering 2480 cM on 25 linkage groups and a genome-wide QTL scan was performed to identify the loci underlying the disease. Two different models was used and compared, cnF2freq (Nettelblad *et al*, 2009) and the Flexible Intercross Analysis (FIA) (Rönnegård *et al*, 2007). Statistical chromosome-wide significance was established by randomization testing using 1000 permutations. A 5 % threshold was set to detect significant QTL and a 20 % threshold to detect suggestive QTL.

Genome-wide thresholds were derived from the chromosome-wide significance levels, using a bonferroni correction (De Koning, 2001)

$$(P_{genome\text{-}wide} = 1 - P_{chromosome\text{-}wide})^{1/r}$$

were r is obtained by dividing the length of a specific chromosome by the length of the genome considered for QTL detection (2480 cM).

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FIA is a variance component model that calculates the covariance between the individuals, allowing for segregation, which will increase the power to detect QTL. We expect that FIA will have a higher power to detect QTL since many alleles are not fixed in this outbred cross. SL chickens originates from BL chickens, which will give us more unexplained residual variation. FIA uses score statistics to save computational time and contrasts are based on gametic origin if independence or segregation and on line origin if fixed (Rönnegård *et al*, 2007).

cnF2freq is a Haley Knott based method that calculates the most probable genotypes with a Hidden Markov Model (HMM). It is more efficient than the original Haley Knott method since it has the capacity to handle 1000s of markers with mixed information content, for which there are no other suitable method available at present (Nettelblad *et al*, 2009). A generalized linear regression model was used including the fixed effects of sex and batch along with additive and dominance coefficient for the putative QTL.

Epistatic interaction analysis was conducted as described by Carlborg *et al* (2006, 2003) and was included to detect QTL without significant marginal effects but for which marginal effects together with interaction effects are sufficient to indicate QTL activity. This method uses a genetic algorithm instead of an exhaustive enumeratic ("step-by-step") approach to search for interacting QTL pairs, which makes it feasible for single processor computers.

Results and discussion

The results obtained with the two methods were highly correlated. Both identified the same genome-wide significant QTL on chromosome 3 ($P < 0.00011$). FIA also reported a genome-wide significant QTL on chromosome 9 ($P < 0.05$) and a suggestive QTL on chromosome 21 ($P < 0.20$). There was no proof of segregation, which would have given FIA increased power to find QTL. cnF2freq reported the QTL on chromosome 9 as suggestive ($P < 0.10$) and did not confirm the suggestive QTL on chromosome 21. Birds that have the disease alleles at the QTL, either on chromosome 3 or chromosome 9, are two times more likely to get the disease (Odds Ratio for each locus = 2.0).

With the epistatic par-wise analysis we confirmed that loci on chromosome 3 and chromosome 9 had a genome-wide significant interaction ($P < 0.05$).

Figure 1 shows that the disease is increased if one locus is heterozygous while the other one is homozygous for the disease allele (allele 2). It also shows a slightly increased incidence, but not significantly different, of the disease when both locus has the homozygous genotype probability for the disease allele or when both locus has the heterozygous state. The groups of animals with either of the homozygous genotypes at both loci have a lower number of individuals compared with the other groups, which might affect the result.

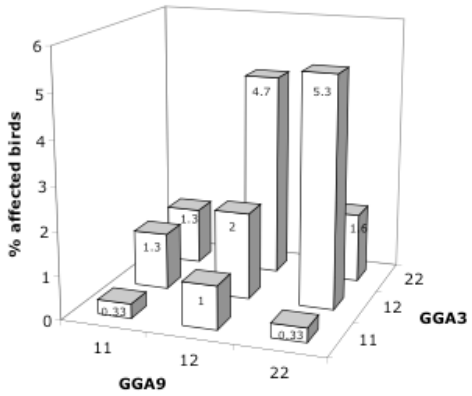

Figure 1. % affected birds for each genotype probability for the loci on chromosome 3 (GGA3) and chromosome 9 (GGA9).

Conclusions

This study identified two significant QTL on chromosomes 3 and 9. The Odds Ratio of developing vitiligo is two when the QTL is present. Interestingly, we also found a significant interaction between these two loci. Further work will be done to narrow down the QTL and search for possible candidate genes in these regions.

References

Carlborg, O., Jacobsson, L., Ahgren P. *et al*. (2006). *Nat Genet* 38: 418-420.

Carlborg, O., Kerje, S., Shutz, K. *et al*. (2003). *Genome Res* 13: 413-421.

De Koning, D.J. (2001). *Doctoral Thesis*.

Nettelblad, C., Holmgren, S., Crooks, L. *et al*. (2009). *BICoB* 307-319.

Rönnegård, L., Besnier, F., Carlborg, Ö. (2007). *Genetics* 178: 2315-2326.

Spritz, R. (2005). *Journal of Dermatological Science*, Volume 41, Issue 1:3-10.